Immunologically Modified Field Effect Transistors for Protein Detection in Biologic Fluids

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Abstract

Field effect transistors (FETs) are solid-state electrical devices with semiconductor channels through which charge carriers migrate and generate current. The application of an electric field proximal to the conductive channel causes a change in current depending on the sign and magnitude of the field. FETs can be modified for protein sensing by deployment of antibodies as receptors on the channel surface to create an immunologically modified FET (immunoFET). Binding of analytes brings a layer of charge proximal to the channel surface, causing modulation of current that is easily detectable, allowing for quantitative detection of unlabeled analytes. The FET design may be scalable to allow for inexpensive, real-time, label-free, point-of-care diagnostic use. The distance between the analyte and the channel is crucial as sensitivity drops off due to counter-ion shielding, which historically was the reasoning behind assessing immunoFETs as infeasible. Bound proteins must therefore be held within a couple nanometers of the channel for successful detection. Following previous work showing successful detection of various analytes in PBS, we present the successful detection of Monokine induced by Interferon γ (MIG/CXCL9), a pro-inflammatory chemoattractant chemokine, in both murine serum and human urine from transplant patients using AlGaN/GaN heterojunction FETs (HFETs) modified with anti-CXCL9 IgG. ImmunoHFETs were modified with IgG antibodies specific to CXCL9 then exposed to human urine of renal transplant recipients. Baselines and changes in conductance were compared to determine levels of CXCL9 in clinical samples. We present the detection of CXCL9 in renal transplant urine at biologically relevant levels and correlated with rejection by renal biopsy. The presented work demonstrates the feasibility of immunoHFET sensor operation in physiologic buffers, and shows the potential to provide real-

time quantification and monitoring of inflammatory mediators, allowing for low-cost and minimally invasive real-time interrogation of graft status.

Acknowledgements

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Nomenclature

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sufonic acid)
APDMES	Aminopropyl dimethylethoxy silane
APTES	(3-Aminopropyl)triethoxysilane
AlGaN	Aluminium Gallium Nitride
BSA	Bovine serum albumin
CCL5	Chemokine Ligand 5 (also known as RANTES)
CXCL9	Monokine induced by Interferon γ (also known as MIG)
ELISA	Enzyme-linked immunosorbent assay
FET	Field effect transistor
GaN	Gallium Nitride
HFET	Heterojunction field effect transistor
HRP	Horseradish Peroxidase
I_{ds}	Source-Drain Current
ICP	Inductively coupled plasma etching
IgG	Immunoglobulin G
ISFET	Ion selective field effect transistor
immunoHFET	Immunologically modified field effect transistor
MIG	Monokine induced by Interferon γ (also known as CXCL9)
MOSFET	Metal oxide field effect transistor
PBS	Phosphate-buffered saline
RANTES	Regulated upon Activated, Normal t-cell Expressed, and Secreted (also known as CCL5)
SA	Streptavidin
SA-HRP	Steptavidin conjugated to horseradish peroxidase
SDS	Sodium dodecyl sulfate
TEA	Triethoxysilane aldehyde
2DEG	2-dimensional electron gas

Introduction

Motivation for Biosensing

Proteins, which have influence over innumerable processes in nature, are involved in host responses to a wide variety of disease formation and defense in the human body. For this reason, it can be diagnostically significant to identify the concentrations of key proteins and may also facilitate the staging of disease progression. Along with many diseases, various pathological immuno-inflammatory states may also be identified by monitoring a specific family of protein molecules called cytokines. These small molecules (5-20kDa) are known to be heavily involved in cell signaling within the immune system and therefore, a great deal of effort has been directed towards better understanding proteins within these pathways [5].

Currently, protein detection and quantification is accomplished by enzyme-linked immunosorbent assay (ELISA), western blot, bicinchoninic acid assay (BCA), etc. and while effective, these techniques require large quantities of costly reagents and are also time and labor intensive. As potentially pivotal devices in the clinic, biosensors have the ability to monitor disease prior to the development of symptoms by quantifying concentrations of specific proteins in an inexpensive and timely manner.

As depicted in Figure 1, biosensors incorporate the use of a bioelement for specific recognition of an analyte (typically an enzyme or antibody) as well as a transducer to convert the binding event into a readable signal (potentially a temperature, conductance, potential, current, or impedance). Transducers used within biosensors can be as diverse as semiconductor or cantilever based devices.

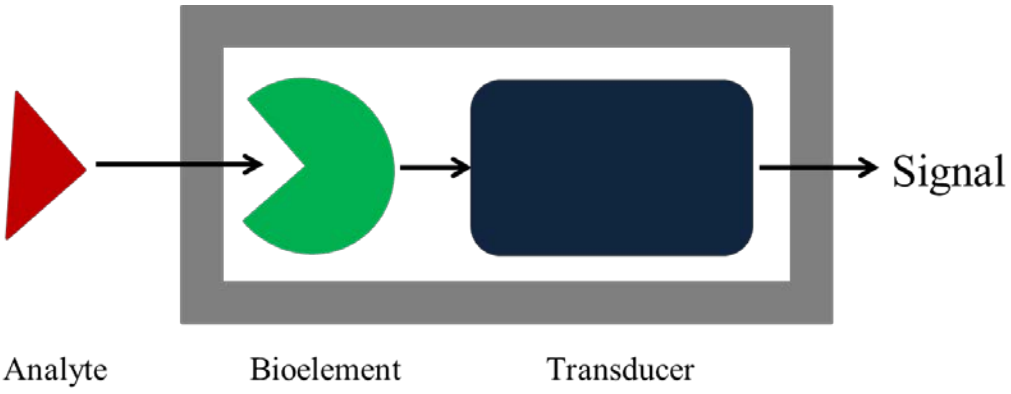

Figure 1: Biosensor technology.

Field Effect Transistors as Biosensors

Typical field effect transistors (FETs) are three-terminal solid-state electrical devices consisting of a source and drain, which are biased to drive current flow, and a gate, used to modulate the current through the application of a bias proximal to the conductive channel. The bias produces an electrical field, which effects charge carrier flow and causes either an increase or decrease in current depending on the sign of the field.

As in metal oxide semiconducting FETs (MOSFETs), the gate allows for current flow if above (for n-type) or below (for p-type) the threshold voltage. The n-type FET, which utilizes electrons as charge carriers, will be discussed from this point on for the purposes of this research. As the bias is altered above the threshold voltage, more charge carriers are drawn into the channel from the doped wells and the device current increases whereas when the gate bias moves closer to the threshold voltage, fewer charge carriers are drawn into the channel and the current is decreased.

As biosensors, FETs utilize a biological affinity element deployed on the sensing surface to bind the analyte of interest and hold it in close proximity. The charge of the bound analyte then imparts an electric field which alters current flow throughout the conducting channel. The

change in current within the channel is measured and correlated to a concentration of the specific analyte in solution. FET-based sensors have been studied extensively since the creation of the ion sensitive FET (ISFET) by Bergveld, et. al. in 1970. Basic ISFET operation is broadly similar to that of MOSFETs except that the gate on the capacitance layer is replaced by enzymes as the affinity elements whose ions provide the current modulation [9,10].

The operational properties of FET biosensors provide potential advantages for protein sensing/detection, including label-free detection, high sensitivity, rapid response, convenient size scalability, and low fabrication costs. By being based on intrinsic properties of analytes, FET biosensors are also platforms for label-free detection. FET biosensors may lead to minimally-invasive point-of-care diagnostics that are faster and less expensive than currently used immunoassay or biopsy.

Classical ImmunoFET Model

The distance between the analyte and the sensing channel is crucial as sensitivity drops off to 6^{th} the power of distance due to counter-ion shielding that occurs in high ionic concentrations (150nM Na^+, pH 7.4), which historically was the reasoning behind assessing the possibility of an immunoFET as infeasible [12]. The distance from the sensing channel for which electrostatic effects persist, the Debye length, is on the order of a few nanometers when working in PBS meaning that beyond this, mobile ions screen out the electric fields of the bound analytes. The classical assessment asserts that the bound analyte will be at a distance equal to the length of an antibody (10-15nm for chemokines) from the sensing surface and thus beyond the Debye length [9, 10].

This model hinges on the idea that antibodies uniformly adsorb onto the sensing surface by the C_H3 region (Figure 3) and orient in a manner that positions the variable domains of the antibody at a distance further than the Debye length. This is represented in Figure 2a, however a more realistic depiction of antibody alignment on an immunoFET can be seen in Figure 2b [7]. Antibodies are known to adsorb to surfaces in a non-uniform manner as shown.

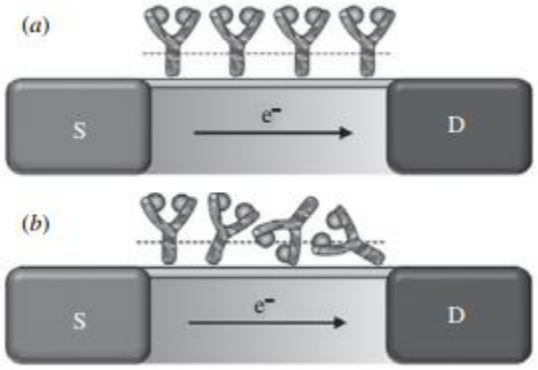

Figure 2: (a) Classical representation of antibodies (Y shapes) deployed on an immunoFET surface. Approximate Debye length position is indicated by the dashed line. **(b)** A more realistic depiction of antibody alignment on an immunoFET. Adapted from Casal, et. al. [7]

The classical assessment also assumes that antibodies are rigid structures when in fact they are highly flexible. Figure 3 depicts a simplified IgG molecule consisting of four polypeptide chains held together by disulfide bonds. Antigen binding is mediated by the variable light (V_L) and variable heavy (V_H) regions while common effector functions are controlled by the constant regions of the antibody. The portions of the antibody that add to the flexibility include the hinge region (the disulfide linkages between constant region 1 and constant region 2) as well as the molecular ball-and-socket region between variable and constant domains. These regions create a large degree of conformational freedom allowing for bending and thus a wide range of distances over which the bound analyte charges can be held from the semiconducting surface.

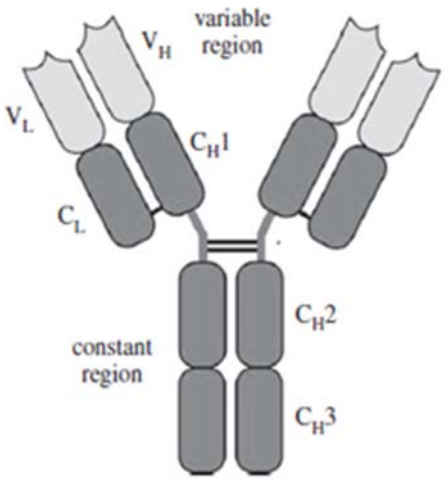

Figure 3: Simplified depiction of IgG Antibody. Adapted from Casal, etl al. [7]

When taken together, the stochastic adsorption along with the flexibility of antibodies leads to a distribution of distances over which the bound analyte charges are held from the sensing surface. A portion of these charges are expected to be within the Debye length in physiological conditions. This indicates that charges may be held in close enough proximity to cause current modulation from source to drain, thus deeming the classical assessment as inconsequential to the development of an immunoFET.

Research Focus and Significance

Provided the theoretical flaws of the classical model of immunoFET function and previous work for detection of various analytes in PBS, the fundamental goal of this work was to develop immunoFET devices for use in physiological solution. Preliminary results for immunoFET function in PBS have been published showing that signal magnitude for a fixed analyte charge is directly related to charge proximity to the sensing channel [7, 28]. The work presented herein focuses on the development and testing of an immunologically modified field

effect transistor for detection of the pro-inflammatory chemokine Monokine induced by Interferon γ (MIG/CXCL9) in physiological solution. As a chemoattractant for cytotoxic T-cells, MIG increases during inflammatory responces from the normal concentrations of 40-100pM to 1-2 orders of magnitude higher, indicating the onset of acute inflammatory response. The monitoring of MIG concentrations in transplant recipients can be used as an early indicator of allograft rejection [3, 4].

This study completed device and ELISA testing for renal transplant recipient urine samples in an effort to further the progress towards the development of an immunoFET for real-time quantification and monitoring of inflammatory mediators, allowing for minimally invasive interrogation of graft status.

Background

HFET Biosensor Development

FETs are traditionally constructed from silicon-based substances, due to the low-cost and high performance, and also doped with impurities that donate mobile electrons for conduction. However, in physiological salt conditions (150mM NaCl), ions leach into this silicon substrate causing changes in the device performance. In order to avoid this, an ion-impermeable heterojunction FET (HFET) platform made of wide-bandgap materials AlGaN/GaN was developed. This technology also allows for the exclusion of doped wells and the gate terminal of MOSFETs. This is possible because the junction between AlGaN (the highly doped supply layer) and GaN (non-doped layer) creates a 2-dimensional electron gas (2DEG) sensitive to changes in the electric potential (Figure 4). Dipole moments are introduced along the Ga-N and Al-N bonds due to strong electronegativity of Nitrogen.

HFETs can be modified to allow for protein sensing by deployment of affinity elements (antibodies) as receptors on the semiconducting channel surface to create an immunologically modified HFET (immunoHFET). Binding of analytes to receptors brings a layer of charge proximal to the channel surface, causing modulation of current that is easily detectable and allowing for quantitative detection of unlabeled analytes.

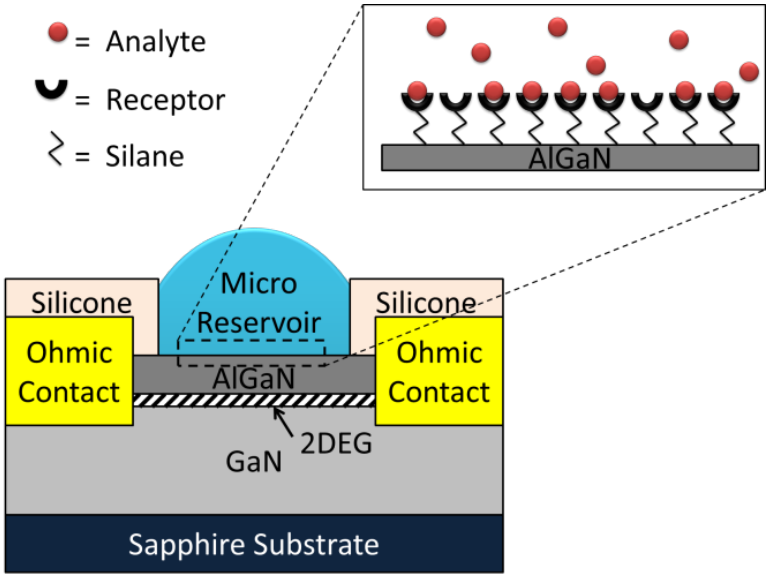

Figure 4: immunoHFET device cross-section.

Detection of Various Analytes in PBS

Monokine induced by Interferon γ (MIG/CXCL9) was and continues to be the primary protein of interest for this work. In previously published works, it has been shown that immunoHFETs have the ability to detect human and murine CXCL9 in PBS [7]. Detection has also been extended to analytes of varying sizes and charges including Chemokine Ligand 5 (CCL5), also known as RANTES (Regulated upon Activated, Normal t-cell Expressed, and Secreted), as well as CXCL10 and streptavidin (SA) in PBS [28]. It has been shown that the

immunoHFET has the ability to detect relatively small proteins of positive charge as well as larger proteins of negative charge: CCL5 (7.8kDa, +5), CXCL9 (11.7kDa, +14), CXCL10 (8.6kDa, +11), and SA (76kDa, -6). A protein has yet to be encountered that is not detectable by immunoHFET technologies. All sensor testing was corroborated by ELISA utilizing a kit purchased from R & D Systems (Minneapolis, MN).

ImmunoHFET Selectivity

In previously published works, it has been shown that immunoHFETs have the ability to selectively bind exceedingly similar analytes in PBS. Shown by Casal, et. al, when functionalized with anti-huMIG antibodies, huMIG was detected but murine MIG was not as seen in Figure 5 [7, 28]. The mature proteins of huMIG and muMIG have similar amino acid lengths (103 vs. 105 respectively) with 72% amino acid sequence homology. This work demonstrates that device signal is driven by receptor specificity i.e. device specificity is determined by the binding specificity of the receptor deployed on the sensing channel.

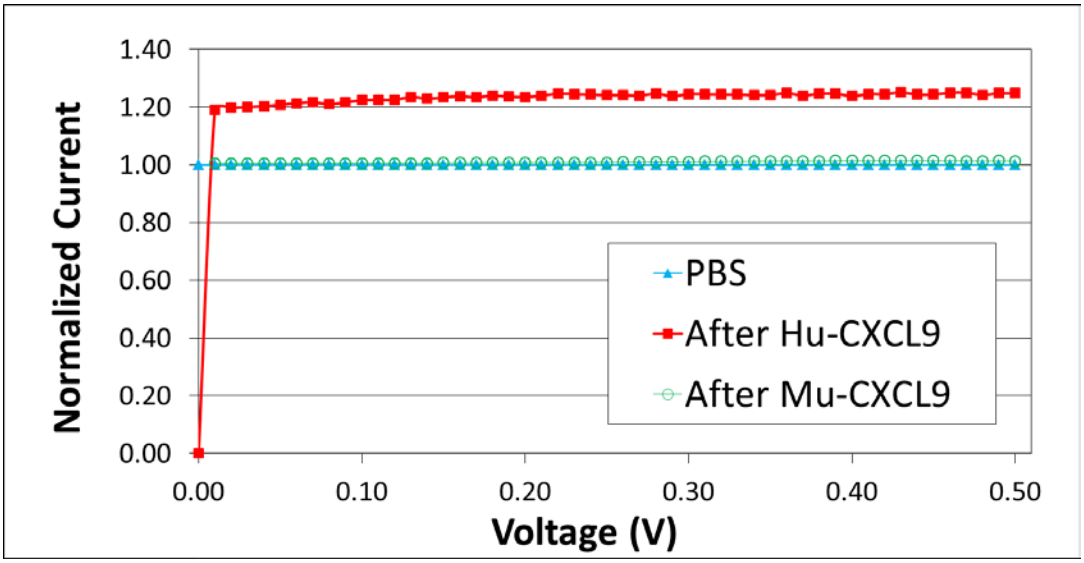

Figure 5: Anti-huMIG IgG Functionalized immunoHFET specificity determined by receptor.

Experimental Procedures

Chemicals

Polyclonal anti-huMIG IgG and a Human MIG ELISA Development Kit were purchased from Peprotech, Inc. (Rocky Hill, NJ). Triethoxysilane aldehyde (TEA) was purchased from United Chemical Technologies (Bristol, PA) to be used as the silane polymer. Steptavidin (SA) conjugated to horseradish peroxidase (SA-HRP) and Dulbecco's phosphate-buffered saline (PBS) containing 150mM NaCl with a pH of 7.4 are from Invitrogen, Inc. (Carlsbad, CA).

HFET Device Construction

As previously published, AlGaN/GaN heterostructures were purchased from CREE, Inc. (Raleigh, NC) and underwent several microfabrication processing techniques (Figure 6). Dry MESA etching was used to remove the exposed AlGaN region prior to electrode pattern photolithography and metal deposition for placement of electrodes. A silicone insulator was then placed over the electrodes to isolate them from the sensing channel.

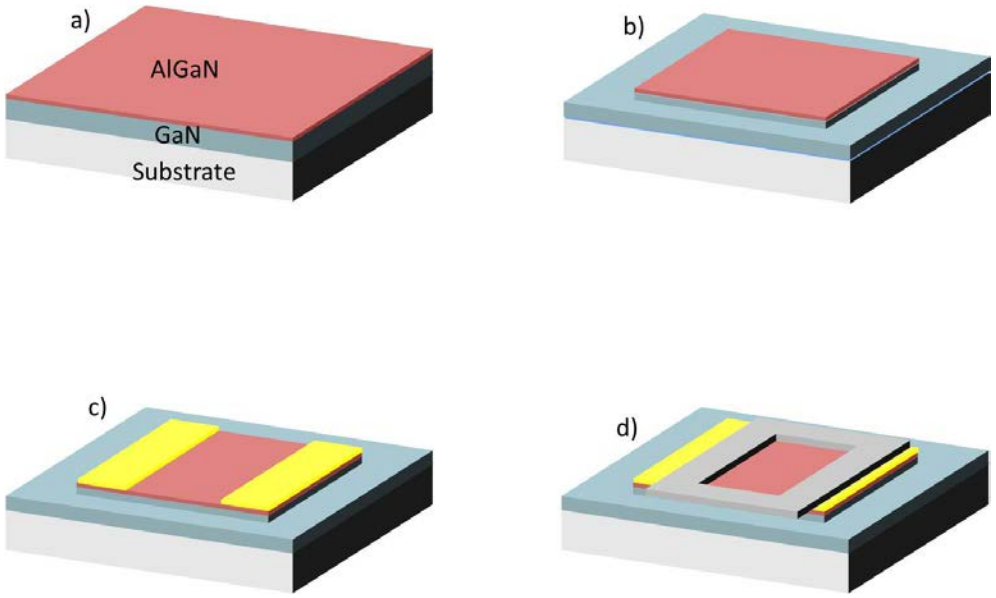

Figure 6: AlGaN/GaN HFET Microfabrication Process

The devices were surface oxidized via inductively coupled plasma treatment (ICP) in an Anatech 600 oxygen plasma chamber for 30 seconds (75Watts, 700mTorr) [5, 8, 7, 22]. One hundred micrometers of the AlGaN layer were recessed in a chloride based ICP so that the threshold voltage of the device was shifted to the -0.5V to +0.5V range [7, 28]. The conducting channel varied from 50µm to 100µm in width and length with a fluid reservoir height of 10-20µm. As described previously, the electrical conductivity between the drain and source was controlled by a chemical gate, formed on the oxide and functionalized with antibodies as receptors for specific analyte recognition.

Transistor Surface Functionalization and Receptor Exposure

Surface functionalization of oxidized AlGaN/GaN HFETS was accomplished by following protocol for APTES deposition but exposing devices to 5% TEA in ethanol overnight within a 50ºC water bath [21]. After a triple rinse in ethanol, devices were treated with 1µg ml^{-1}

of receptor anti-huMIG IgG for 1 hour at 37ºC. The device was then triple rinsed in PBS and Tween 20 (0.05%) prior to sample exposure and electrical measurements.

Electrical Measurements and Sample Exposure

As previously described, three-terminal (source, drain, and gate) current-voltage characteristics of AlGaN/GaN HFETs were measured using an Agilent 4156C semiconductor parameter analyzer at room temperature. The I_{ds} was modulated by the gate bias to the order of $1\mu A \cdot mm^{-1}$ for detection. This was done to ensure that the device was operating in the subthreshold regime in order to maximize sensitivity [22]. The baseline measurement was first taken for the device by exposing to PBS only. The device was then incubated for 5 minutes in15µl of human renal transplant recipient urine at the time of diagnostic renal transplant biopsy, after which devices were washed three rounds in PBS. The I_{ds} was then measured for a second time and the changes in I_{ds} before and after analyte binding showed how the device behavior was altered by the analyte binding event [5].

Immunosorbent Assay

Enzyme-linked immunosorbent assays (ELISAs) were performed to corroborate electrical sensor data with anti-huMIG. The 96-well Nunc Maxisorb ELISA plates were incubated with $1\mu g\ ml^{-1}$ capture antibody anti-huMIG overnight then blocked with sterile filtered 1% bovine serum albumin (BSA) in PBS for 1h before exposure to 100µl of standards and samples for 2h. Standards ranging from 10 to 5000 pg/ml along with a background (no primary antibody) and negative control (PBS only) were used for comparison. Wells were then exposed to the detection antibody anti-huMIG for 2h prior to incubating with avidin-HRP Conjugate (1:2000 in diluent)

for 30 minutes. Wells were rinsed four times with 0.05% Tween-20 in PBS after each previous step and then incubated with ABTS liquid substrate solution for 15 minutes. The stop solution of 1% sodium dodecyl sulfate (SDS) was added and the absorbances of the reacted solutions were measured in the Victor X3 Plate Reader at 405nm.

Results

All data was collected from testing done on the same immunoHFET sensor. We have provided preliminary results for immunoHFETs operating in physiological solution, using an ion-impermeable platform (AlGaN/GaN HFET) and show signal magnitude for a fixed analyte charge to be directly related to charge proximity to the sensing channel [7]. In work described herein, we have presented the successful detection of the inflammatory chemokine CXCL9 in both murine serum and human renal transplant patient urine using immunoHFET modified with anti-huMIG. The presented work demonstrates preliminary results of the feasibility of immunoHFET sensor operation in physiologic buffers as an early step in the translation of this technology to human clinical applications. Many opportunities for increased sensitivity and optimization exist for this device, which will be further investigated.

Detection of CXCL9 in Murine Serum

After detection of huMIG in PBS, preliminary studies were completed for detection of huMIG in a complex physiologic buffer of murine serum [28]. Murine serum, a much higher sample fluid complexity as compared to PBS, contains all proteins not used in coagulation as well as hormones, electrolytes, and various antibodies and antigens. Murine serum was doped with 100ng/ml of huMIG. Anti-huMIG functionalized immunoHFETs were incubated with 1%

BSA sterile filtered in PBS for 1 hour in order to block non-specific binding. Three-terminal current-voltage characteristics were measured as described previously. Figure 7 shows the electrical measurements for baseline (PBS only), undoped murine serum, as well as murine serum doped with huMIG. These results act to provide proof of concept that the immunoHFET has the ability to detect huMIG in a complex physiological solution, murine serum.

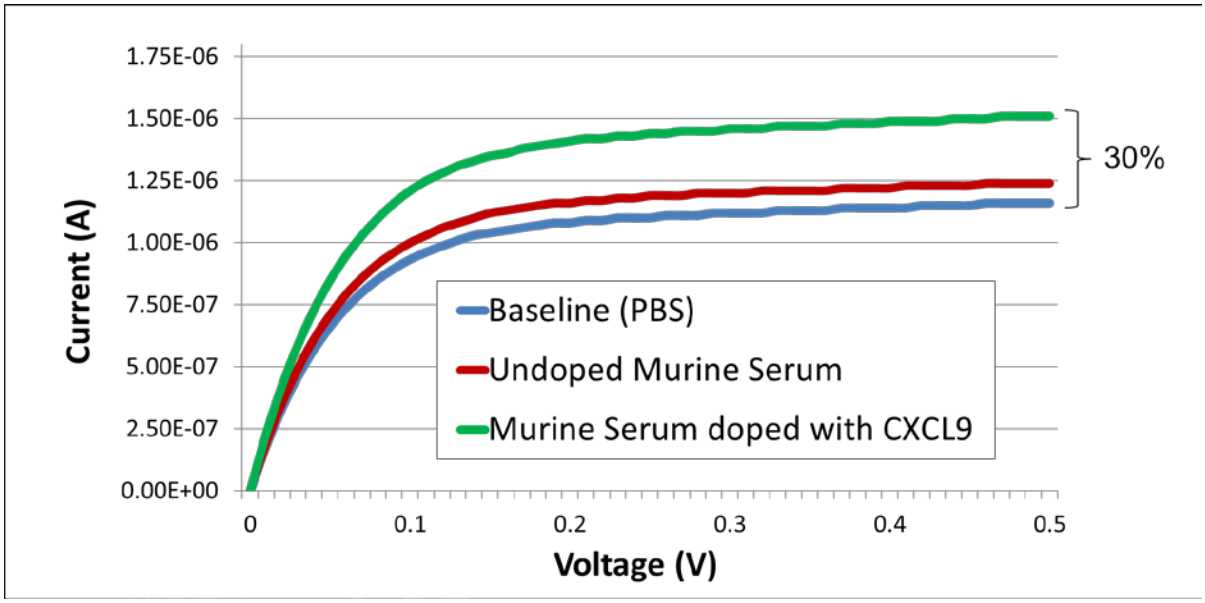

Figure 7: ImmunoHFET detection of huMIG in murine serum. Adapted from Casal, et. al. [28].

In order to corroborate the interaction of huMIG with anti-huMIG IgG deployed on the immunoHFET sensor surface, ELISAs were completed with a kit purchased from R& D Systems (Minneapolis, MN), the results of which can be seen in Figure 8 [28].

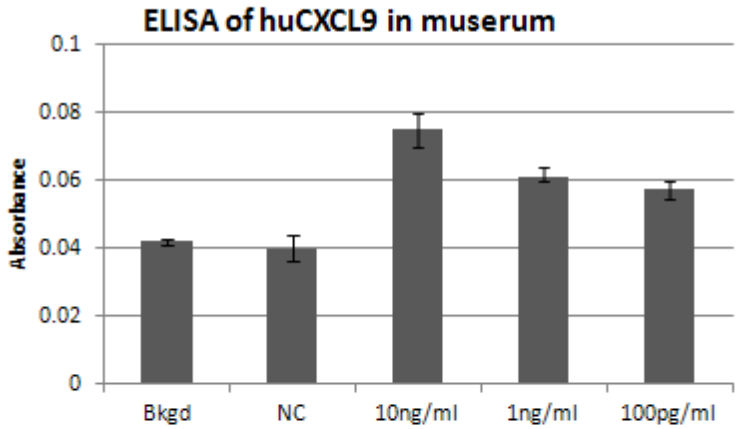

Figure 8: ELISA Corroboration for detection of huMIG in murine serum. Adapted from Casal, et. al. [28].

CXCL9 Detection in Human Renal Transplant Recipient Urine

Three-terminal current-voltage characteristics were measured as described previously. Preliminary immunoHFET sensor response to urine samples from both a rejecting and a non-rejecting patients. Figure 9 shows early electrical measurements for urine samples from one rejecting patient (patient 4) and one non-rejecting patient (patient 5) as compared to baseline measurements (initially blinded to patient allograft status).

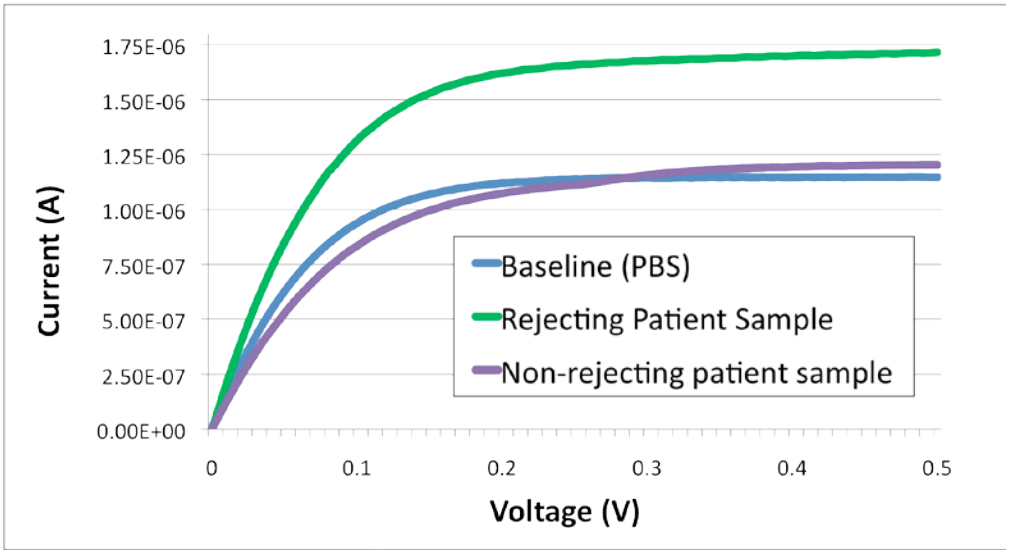

Figure 9: CXCL9 detection in urine of renal transplant patients.

As clinical samples were collected, more sensor testing was completed, however issues began to arise with the results being obtained. Triplicates of each test were completed for one rejecting and two non-rejecting patient samples. Table 1 shows the average percent change in signal along with the standard deviation between triplicates.

Table 1: Sensor Testing Data

Patient	Rejection Status	Average Percent Change in Signal	Standard Deviation
13	Non-Rejecting	60%	11.65
15	Rejecting	53%	11.95
18	Non-Rejecting	52%	36.86

Throughout all testing, one sensor was utilized and subjected to surface modification for each test performed. The act of stripping molecules from the surface of the immunoHFET and re-oxidizing the AlGaN surface via inductively coupled plasma (ICP) reactive ion etching may

have surface damaging effects. When performed a minimal number of times, ICP at 75 Watts and 700mTorr for 30 seconds would cause negligible damage to the surface, however the sensor used throughout testing had undergone countless iterations of this process and was likely to have accrued damage over the past years of testing.

As shown by Fang, et. al., if damage free etching is desired, photoenhanced chemical (PEC) wet etching would be the most effective technology for surface functionalization, while hybrid ICP/PEC etching can be used for applications requiring high etch rate and damage-free surfaces [23]. However moving forward, surface damage by etching will not be a concern as it is desired to move toward one-use disposable devices.

Another possibility for the introduction of error could be disregarding the use of 1% BSA sterile filtered in PBS in sensor testing. This blocking buffer was used throughout ELISAs after deposition of capture antibody anti-huMIG in order to block the remaining binding surface from nonspecific binding of antibodies and also in the sensing of huMIG in murine serum (Figure 7). This reagent however was not used in sensor testing with human patient urine samples and may be the reasoning behind the lack of correlation between sensor data as compared to biopsy rejection status.

ELISA Corroboration

ELISAs were performed as described above in coordination with protocol provided by PeproTech, Inc. (Rocky Hill, NJ) in order to corroborate electrical sensor data. The procedure provided by PeproTech, Inc. was explicitly followed including all reconstitution and storage methods. The first set of ELISA data (patients 1-8, 9/19/2013) proved to be promising and can be seen in Figure 11 while initial standard absorbance readings can be found within Figure 10.

As compared to Figure 9, patient 4 (rejecting) showed increased levels of CXCL9 in the data obtained by ELISA while patient 5 (non-rejecting) did not. Increased levels of CXCL9 seen for patients 1 and 2 also coordinate with rejection status as confirmed by biopsy.

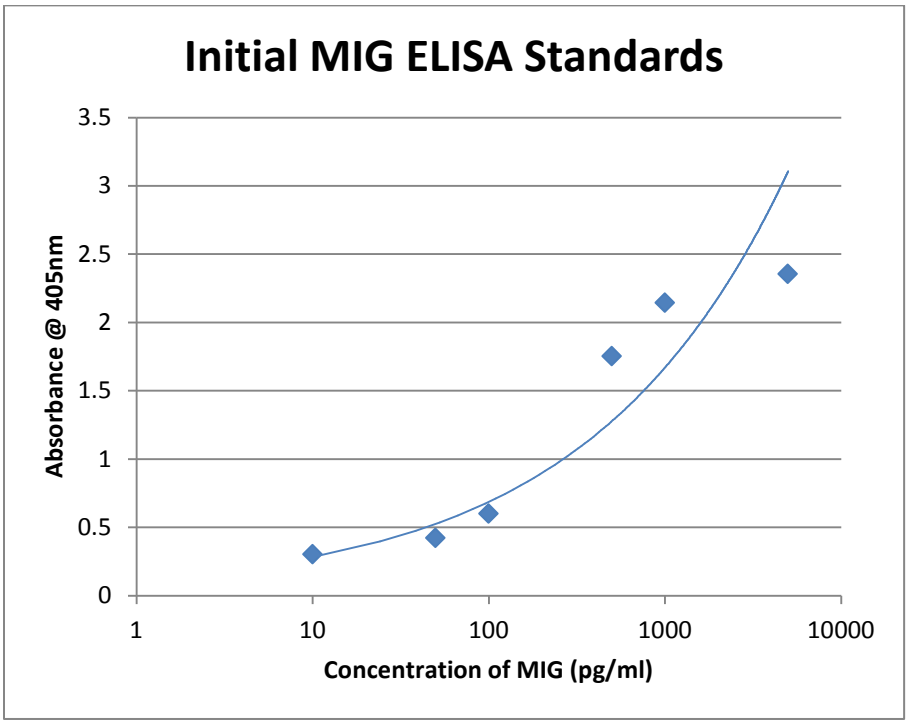

Figure 10: Initial MIG ELISA Standards

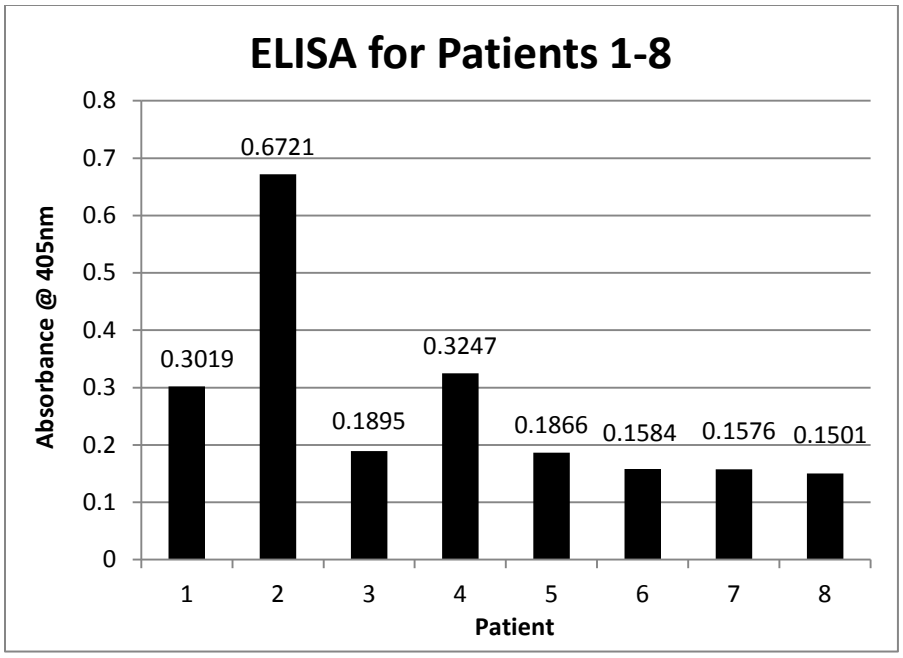

Figure 11: ELISA Results for Patients 1-8

After a successful first ELISA with the Peprotech kit, we began to see complications with the results. Although protocol states that when stored at -20ºC, aliquots are stable for up to 2 years, it can be seen in Figure 12 that as time passed, problems arose with the reagents and thus the results of the ELISA suffered. Since the initial creation of the standards on 9/19/2013, the absorbances decreased and the avidin-HRP conjugate actually became unusable.

Avidin-HRP was tested prior to ELISAs by adding 100µl to 100µl of ABTS substrate. If no immediate color change occurred, streptavidin-HRP (1:2000 in diluent) was utilized with TMP/H_2O_2 substrate and sulfuric acid stop solution. This occurred for the ELISA completed on 11/21/2013, hence the vastly different absorbances.

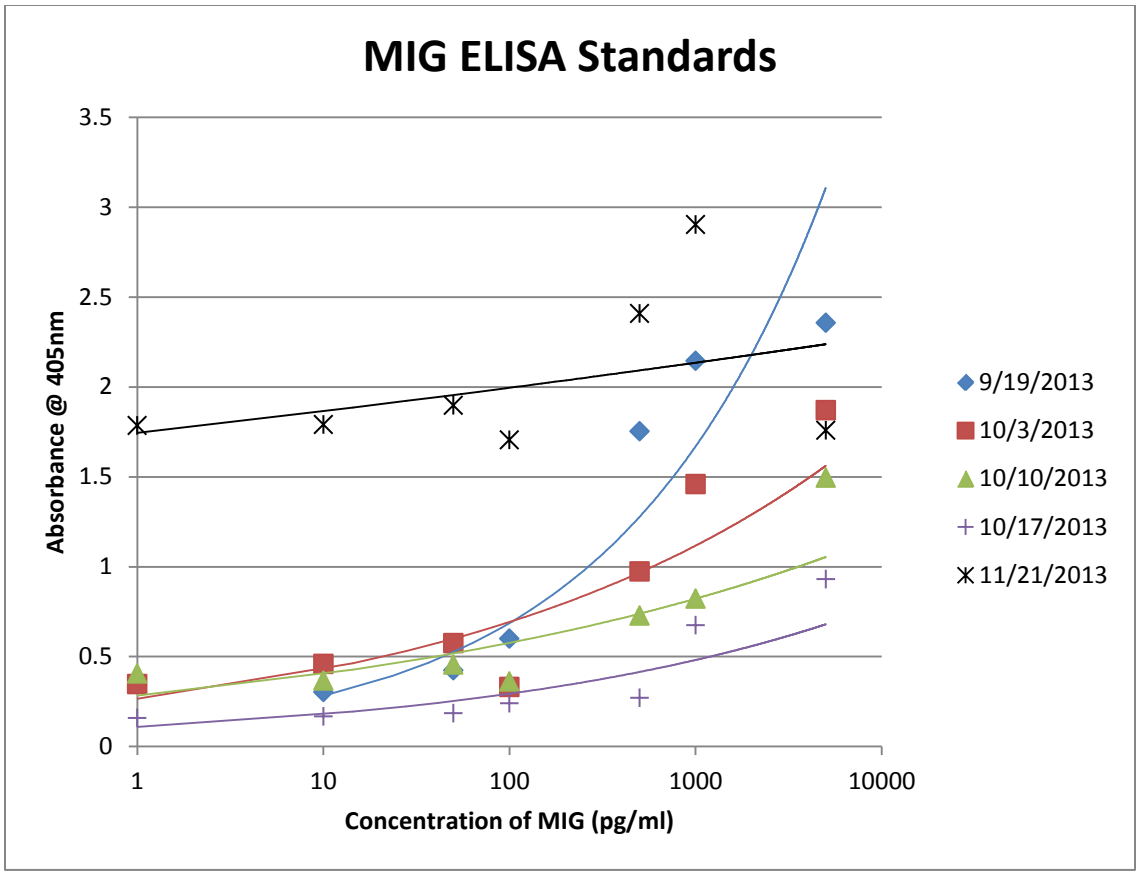

Figure 12: ELISA Standards (MIG) for each ELISA test performed

Future Recommendations

Bovine Serum Albumin in Sensor Testing

As discussed above, 1% BSA sterile filtered in PBS was utilized in detection of huMIG in murine serum, shown in Figure 7, to block non-specific binding after the deposition of primary anti-huMIG IgG antibodies. This blocking buffer was not used in any other sensor testing, however it could be beneficial to know the effects of its use. By going back and implementing the use of this blocking buffer in testing known concentrations of huMIG in PBS, we would be able to identify if this step indeed caused the issues seen in sensor testing.

ELISA Kit

An ELISA kit and protocol from R & D Systems (Minneapolis, MN) was utilized by Casal et. al. to corroborate immunoHFET sensor data in PBS [7,28] and was also used by Hrick et. al. and shown to be successful in the detection of CXCL9 in human urine. In moving forward with ELISA corroboration for sensor data, this same kit should be used as opposed to the less expensive kit purchased from Peprotech, Inc. (Rocky Hill, NJ). Although there are only slight differences in reagents and protocol from the currently used kit, it is expected to provide much more appropriate results in human urine. Renal transplant recipient urine samples should be re-tested using this kit for corroboration with sensor data.

Silane Film Thickness

The sensor used was not engineered to minimize analyte-to-channel distance and thus maximize sensitivity. One key factor that affects this distance in immunoHFET construction is the choice of silane. The sensor used throughout this work incorporates TEA in fabrication, which is a trivalent silane that is similar to (3-Aminopropyl)triethoxysilane (APTES). In using a trivalent silane, it is expected that the height of the silane layer would be greater than the height when utilizing a monovalent silane because of the likelihood to form meshworks as shown in Figure 13a. These meshworks arise as a result of APTES-type silane monomers having the ability to form infinite, cross-linked siloxane polymer lattices.

Constructing the immunoHFET exploiting a monovalent silane with structure similar to aminopropyl dimethylethoxy silane (APDMES) would allow for a highly ordered monolayer to be formed. APDMES-type silanes are unable to polymerize into extensive networks because of only being able to form siloxane dimers or linkages to oxides. The decreased thickness of

monovalent APDMES as compared to trivalent APTES can be seen within Figure 14. Moving forward, an APDMES-type silane with terminal aldehyde group should be considered in surface functionalization to move toward increased sensitivity.

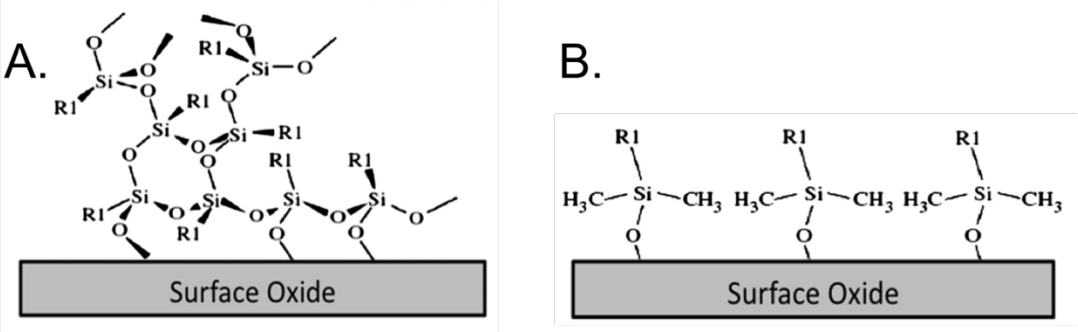

Figure 13: Conceptual illustration of APTES (a) and APDMES (b).

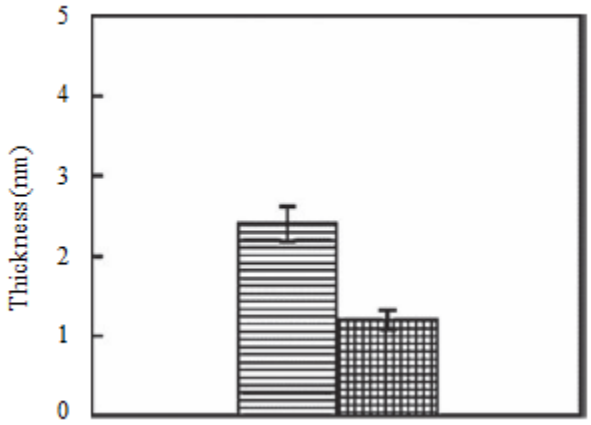

Figure 14: Film thickness of trivalent (APTES) and monovalent (APDMES) silane polymer films. Adapted from Bhushan et al [25]

Engineered Antibodies as Receptors

The work presented herein utilized intact IgG antibodies (~10-15nm, 150-170kDa) as immunoHFET affinity elements deployed on the sensing surface. By reducing the size of the

receptor used in device construction, the distance from sensing channel to analyte would be decreased, thus increasing sensitivity.

The isolation of specific epitope regions of an antibody is possible and would be likely to greatly decrease the distance from bound analyte to sensing channel of the immunoHFET device. Single-chain fragment variable antibody fragments (scFvs) are fused proteins made of the heavy and light variable chains of IgG antibodies and are approximately half the size of an intact IgG antibody at 5-6nm. Single domain antibody fragments known as variable heavy-heavy (VHH) fragments are even smaller at 2-4nm [26].

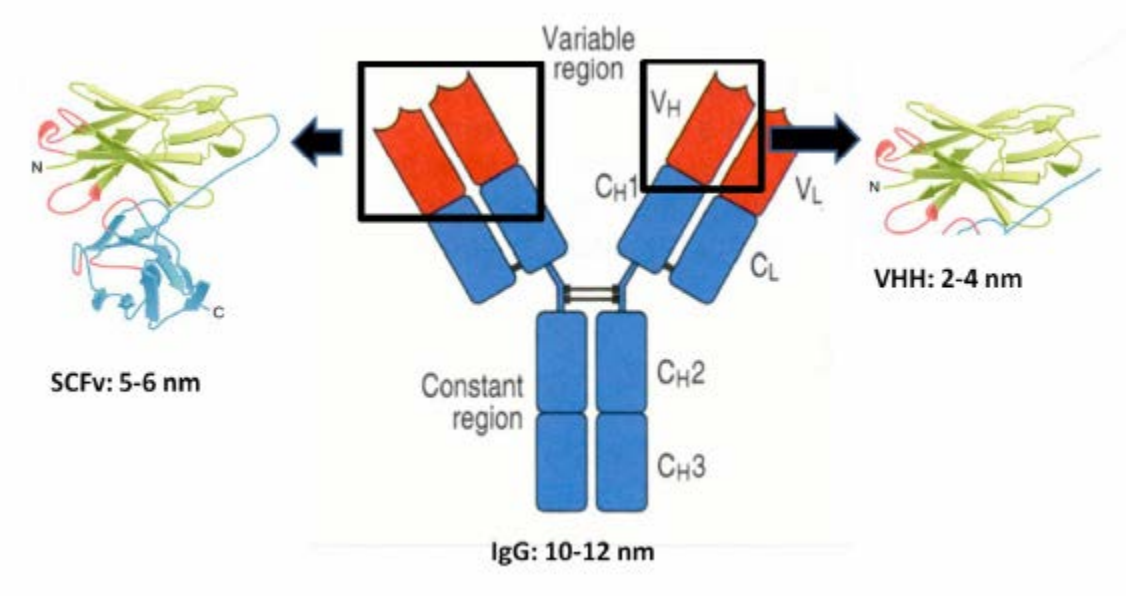

Figure 15: Depiction of intact IgG antibody as well as single-chain fragment variable (scFv) and variable heavy-heavy (VHH) fragmented antibodies adapted from Casal, et al. [28]

Both of these identified antibody fragment options retain full analyte recognition properties even though they have the possibility to be as small as 10% the mass of intact antibodies [26].

As discussed by Casal, et. al., there exists the possibility to further obviate the concerns of ion shielding for immunoFETs by altering the protein topography via scanning circular permutagenesis (CP) [27, 28]. CP connects the N- and C- termini of a protein via a peptide linker and allows for the introduction of newly placed N- and C- termini at a specified location in the protein sequence. In doing so, it is possible to control the analyte-sensing channel distance as well as orientation as shown in Figure 16 [5]. The depictions in this figure show CP variants of scFvs covalently attached to the sensing surface at their N-terminus. By this, it is possible to place the site of analyte binding at a controlled (either greater or lesser) proximity to the sensing surface. Currently, the sensitivity for successful detection of huMIG in physiological conditions is present and for this reason, circular permutagenesis is not required however, if problems arise in obtaining appropriate sensitivity, the possibility exists.

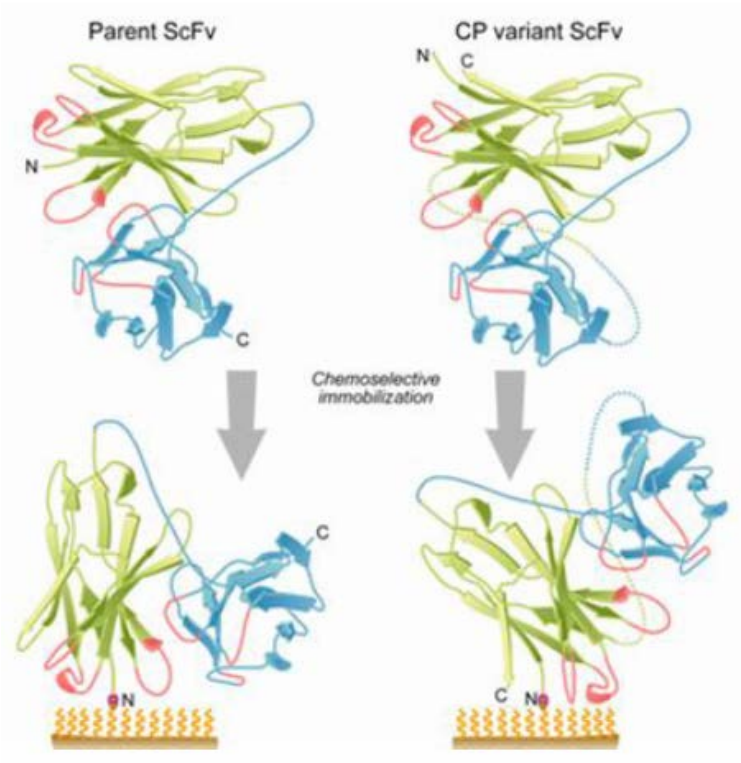

Figure 16: Circular Permutagenesis of a scFv Adapted from Gupta, et. al. [5].

The engineered antibody receptors suggested here suggest further control of the distance from sensing surface to bound analyte. As previously discussed, the control of this distance should be useful in the optimization of sensor sensitivity and thus the ability to detect transplant rejection at an earlier stage in the immuno response.

Conclusions

This work takes the previous knowledge of immunoHFET feasibility to detect analytes in PBS and expands into physiological salt conditions. We have presented the successful detection of the inflammatory chemokine CXCL9 in both murine serum as well as human urine from transplant patients using an immunoHFET modified with anti-CXCL9 IgG. CXCL9 was detected in renal transplant urine in biologically relevant levels, corroborated with ELISA, and correlated with rejection by renal biopsy. The presented work demonstrates preliminary results of the feasibility of immunoHFET sensor operation in physiologic buffers and represents an early step in the translation of this technology to human clinical applications. The immunoHFET design may be scalable to allow for the potential to provide real-time quantification and monitoring of inflammatory mediators, thus allowing for minimally invasive interrogation of graft status at the bedside.

The continuance of testing in complex physiologic environments (transplant patient urine and serum) will allow for increased evidence of the feasibility of immunoHFET detection of analytes in in vivo conditions. It is anticipated that when taking into consideration the use of BSA as a blocking buffer, similar results to preliminary testing (Figure 9) will be reached in testing samples from both patients undergoing acute rejection episodes as well as non-rejecting

patients. The differential CXCL9 levels should be confirmed by ELISA with the use of a kit purchased from R & D Systems (Minneapolis, MN) and initially blinded to patient allograft status.

Many areas of optimization exist within the composition of the immunoHFET device including the choice of receptor and polymer film used in fabrication. The sensors used in preliminary studies were not engineered to minimize the analyte-to-sensing channel distance thus maximizing the sensitivity. This critical parameter may be addressed by multiple means, including investigating the composition of the sensor interface and silane used as well as the use of antibody fragments or circular permuted antibodies as receptors. Increasing the sensitivity of the immunoHFET allows for earlier detection of CXCL9 and therefore earlier indication of renal transplant rejection.

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